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Effects of Heavy Metal Pollution on Oak Leaf Microorganisms

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During the growing season, comparisons were made of the leaf surface microflora of (i) two groups of mature oak trees, one in the vicinity of a smelting complex contaminated by heavy metals and the other at a relatively uncontaminated site, and (ii) two groups of oak saplings at the uncontaminated site, one of which was sprayed with zinc, lead, and cadmium to simulate the heavy metal pollution from the smelter without the complicating effects of other pollutants. Total viable counts of bacteria, yeasts, and filamentous fungi (isolated by leaf washing) were generally little affected by the spraying treatment, whereas polluted leaves of mature trees supported fewer bacteria compared with leaves of mature trees at the uncontaminated site. Numbers of pigmented yeasts were lower on polluted oaks and on metal-dosed saplings compared with their respective controls. Polluted leaves of mature trees supported both greater numbers of *Aureobasidium pullulans* and *Cladosporium* spp. and a greater percentage of metal-tolerant fungi compared with oak leaves at the uncontaminated site. There were no significant overall differences in the degree of mycelial growth between the two groups of saplings or the mature trees.

Despite concern in recent years over the environmental impact of heavy metals, there are few studies on the effects of these pollutants on microorganisms in their natural environment (2, 3). One such area of contamination of aerial plant surfaces occurs in the vicinity of a smelting complex at Avonmouth, near Bristol, England (11, 12). Leaf surfaces normally provide distinct habitats for bacteria, yeasts, and filamentous fungi (10). On pine saplings and cabbages grown in pots and exposed to contamination at Avonmouth, there were reductions both in the number and diversity of these phylloplane microorganisms compared with an unpolluted site (7). In contrast, overall numbers of phylloplane microorganisms on *Lolium perenne* (ryegrass) growing in the vicinity of the smelting complex were surprisingly little affected by heavy metal pollution, although differences in metal tolerance between different groups of microorganisms were observed (4).

The phylloplane microflora changes both quantitatively and qualitatively with time (6, 8, 9), and at Avonmouth will also be subjected to a cumulatively greater degree of metal contamination during the course of the season. The purpose of the present study was therefore to compare the numbers, species composition, and relative metal tolerances of the microflora of oak leaves at Avonmouth, the polluted site, with those of oak leaves at Long Ashton, an unpolluted control site, during the growing season. In view of the large number of other industries (e.g., chemical and fertilizer production) at Avonmouth producing a variety of pollutants, such as sulfur dioxide and oxides of nitrogen, any difference in the phylloplane microflora between here and Long Ashton may not be entirely attributable to heavy metals. To overcome this problem, a further control was necessary in addition to direct comparisons between the polluted and unpolluted sites. This consisted of a parallel study in which the microflora of two groups of oak saplings placed at Long Ashton were compared, with one group being sprayed with zinc, lead, and cadmium to simulate the heavy metal pollution at Avonmouth without the complicating effects of other pollutants.

MATERIALS AND METHODS

Oak saplings investigation. Acorns were collected during the winter of 1976-77 and germinated under greenhouse conditions. During the following spring, 60 potted oak saplings (1 sapling per pot) were moved out to a field in the grounds of Long Ashton Research Station (Ordnance Survey map reference ST 532705), a site relatively uncontaminated by heavy metals. These saplings were randomly assigned to two groups placed about 5 m apart, with one group being maintained as a control and the other being artificially dosed with heavy metals (see below). For sampling purposes, these two treatments were each randomly subdivided into three sets of 10 saplings.

Dosing procedure. The method used for spraying the oak saplings with heavy metals was based on the technique of Gingell et al. (7). Zinc oxide (100 g), lead monoxide (50 g), and cadmium oxide (1.25 g) were

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added to 1 liter of distilled water (stock suspension), and this was stirred overnight by using a magnetic stirrer to allow full dispersion. Two drops of Triton X-100 (BDH Chemicals, Ltd.), a wetting agent, was added to every 100 ml to facilitate the spreading of the suspension over the leaf surface. Spraying was carried out by using a glass atomizer operated from a portable compressed-air source at approximately 15 lb/in^2 (1.03×10^5 Pa). Approximately 2 ml of suspension was sprayed onto four saplings at a time. Care was taken to ensure as even a coating as possible over the leaf surface. Spraying was carried out on 26 May 1978, and a booster spray was applied on 6 July 1978 to maintain the metal contamination at high levels similar to those at the polluted site.

Mature oaks investigation. The oaks chosen to represent polluted conditions consisted of a single group of trees 0.5 km east-southeast of the Avonmouth smelting complex (Ordnance Survey map reference ST 533789). For sampling purposes, the site was randomly subdivided into three sets of three trees. Since no pure stand of oaks existed at Long Ashton, three individual oak trees within about 100 m of each other were selected to represent the control site.

Sampling procedure. Ten leaves were collected at random from the three sets of saplings, mature trees, or single trees within each site or treatment. The same sets were used on successive sampling occasions. Sampling of both saplings and mature trees took place on 31 May, 19 July, and 21 September 1978. In the case of the mature trees, but not of oak saplings, there were sufficient senescent leaves available to allow a further sampling on 17 November 1978. At the same time, a sample of recently fallen leaves was also collected from the litter beneath each tree or set of trees. The leaf samples were transported back to the laboratory in paper bags.

By using a circular aluminum punch (diameter, 1 cm), a pair of leaf disks was punched from each of the 10 leaves comprising a sample. One set of disks from each pair was retained for heavy metal analysis, and the other set was used in the microbiological assay. Similar disks were cut from three more randomly selected leaves and retained for direct observation. The leaf punch was wiped with sterile Decon 90 (Decon Laboratories Ltd., Brighton, England), rinsed in sterile water, and then flamed in alcohol between samples to minimize cross-contamination.

Microbiological assay. The 10 leaf disks for microbiological assay were placed in a universal bottle containing 10 ml of sterile distilled water with 5-g glass beads (approximately 3 mm in diameter) and shaken for 10 min on a wrist-action shaker. A dilution series was prepared. Samples (0.1 ml) of the original suspension and subsequent dilutions were inoculated onto the surfaces of plates of tryptic soy agar (TSA; 3 g of tryptic soy broth [Difco 0370], plus 20 g of agar per liter) and TSA amended with a combination of cadmium, zinc, and lead (as nitrates) at concentrations of 100, 400, and 800 mg/liter to study the relative metal tolerance of microbial isolates (4). Three replicate plates were prepared for each dilution. The inoculated plates were inverted and incubated at ambient temperature (approximately 20°C) for 4 to 15 days before examination, allowing for the slower growth rate of colonies on metal-supplemented media. It was possible to distinguish and enumerate the predominant genera of filamentous fungi but not of yeasts, from observations based on colonial morphology. Nonpigmented and pigmented yeasts were enumerated, and representative colonies of the latter were tested for ballistospore production. The predominant bacterial isolates were subcultured and identified to generic level, where possible, from observations based on pigmentation, cell morphology, and motility.

Direct observation of fungi. The three leaf disks from each sample for direct observation were placed in 4% (wt/vol) chloral hydrate solution. After clearing, each disk was washed in tap water, stained in phenyl acetic aniline blue for about 7 min (4), mounted in water, and observed with a light microscope (field size, 0.004 cm^2). The number of times, n, that fungal hyphae intersected two crosslines of known length, H, was recorded for each of 10 fields, randomly selected on one surface. The mean length of mycelium per unit area, L, was then calculated from the formula $L = (\pi n)/(2H)$ (14). This procedure was then repeated for the other surface.

Heavy metal analysis. Leaf tissue for heavy metal analysis was treated as follows. Each sample, consisting of 10 leaf disks, was dried overnight at 60°C, soaked for about 24 h in 2 ml of concentrated Analar HNO_3 and then heated until fully digested. The digests were made up to 10 ml with deionized water and analyzed for zinc, lead, and cadmium by using an atomic absorption spectrophotometer (Varian Techtron AA6).

Data analysis. The data for length of mycelium, numbers of microorganisms, and percentage of tolerant isolates (calculated as the ratio of the mean number of colonies present on metal-supplemented media to the mean number present on the corresponding metal-free media) were subjected to a two-way analysis of variance. This was a repeated-measures design (15) since the same three sets of plants were sampled on successive occasions. Each set of data was subjected to a logarithmic transformation before analysis so that it should approximate more closely to a normal distribution (13). For statistical purposes, the numbers of microorganisms on the leaves versus litter in November were considered as two distinct sampling times. In microbiological terms it is reasonable to assume that the leaves collected after abscission were likely to be in a more advanced state of decomposition than the senescent leaves remaining on the trees.

RESULTS

Heavy metal concentrations. On every occasion, levels of zinc, lead, and cadmium were far greater on artificially treated saplings compared with the controls (Table 1) and on mature oaks at the polluted site compared with those at the control site (Table 2). On the dosed plants, somewhat higher levels of heavy metal contamination were obtained compared with the oaks at the polluted site. In the case of the artificially dosed oaks, there was a fall in levels during the season, and by September, they were of the same order of magnitude as those at the polluted

TABLE 1. *Mean levels (± the standard error) of zinc, lead, and cadmium on oak saplings at the control site*

Sampling time	Amt of heavy metal (μg/g [dry wt])					
	Controls			Artificially dosed trees		
	Zn	Pb	Cd	Zn	Pb	Cd
May	37 ± 13	8 ± 8	—[a]	12,200 ± 1,000	7,500 ± 1,000	123 ± 16
July	35 ± 3	13 ± 5	—	5,300 ± 200	2,800 ± 230	23 ± 5
September	59 ± 9	18 ± 3	0.2 ± 0.1	1,050 ± 560	950 ± 180	7 ± 4.5

[a] —, Not detected.

TABLE 2. *Mean levels (± the standard error) of zinc, lead, and cadmium on mature oak trees at the control and the polluted sites*

Sampling time	Amt of heavy metal (μg/g [dry wt])					
	Control site			Polluted site		
	Zn	Pb	Cd	Zn	Pb	Cd
May	15 ± 15	10 ± 10	—[a]	430 ± 12	350 ± 14	0.8 ± 0.8
July	11 ± 7	10 ± 1	—	1,210 ± 89	830 ± 80	11 ± 1
September	22 ± 10	8 ± 2	0.2 ± 0.1	1,120 ± 63	1,600 ± 120	14 ± 1
November (leaves)	58 ± 32	43 ± 17	1.7 ± 0.3	1,700 ± 280	2,600 ± 370	37 ± 8
November (litter)	54 ± 17	24 ± 19	1.0 ± 0.6	1,660 ± 150	2,500 ± 270	35 ± 5

[a] —, Not dectected.

site. On mature oaks at the polluted site, levels of zinc exceeded levels of lead in May and July, but not in September and November. On both the control saplings and mature trees at the unpolluted site, there was a small accumulation of heavy metals over the season.

Microorganisms isolated from oak saplings. Bacteria, yeasts, and filamentous fungi were isolated on metal-free TSA, whereas metal-supplemented TSA only supported the growth of fungi (Table 3). On no occasion were bacteria isolated from this latter medium. There was a large increase in the total number of bacteria isolated over the season but no overall difference between the number isolated from uncontaminated saplings and the number treated with heavy metals. *Erwinia* spp. tended to predominate among the bacterial flora on both treatments and on every sampling occasion. Other bacteria isolated included *Xanthomonas* spp., *Pseudomonas* spp., *Flavobacterium* spp., and various orange chromogens, and these also did not appear to be significantly affected by the spraying treatment.

As with the bacteria, there were highly significant differences in the total number of fungal isolates on different sampling occasions. Total numbers of filamentous fungi and *Cladosporium* spp. (the predominant genus) were not affected by heavy metal contamination, and there were also no significant differences between dosed and uncontaminated saplings in terms of the percentages of these isolates able to grow on metal-supplemented TSA. Conversely, although the total number of *Aureobasidium pullulans* (de Bary) Arnaud was also unaffected by heavy metal contamination, the dosed plants supported a significantly higher percentage of metal-tolerant isolates.

The total number of yeasts (excluding *A. pullulans*) and the nonpigmented group were unaffected by the heavy metal dosing treatment. However, numbers of pigmented yeasts were significantly reduced on saplings sprayed with heavy metals. This difference between the two treatments was itself significantly reduced in September compared with July. Subculturing of representative colonies of these pigmented yeasts and testing for ballistospore production suggested that most of them were *Sporobolomyces roseus* Kl. and Van Niel. The total percentage of metal-tolerant yeasts did not differ significantly between treatments. No attempt was made to partition these tolerant yeasts on metal-supplemented TSA into pigmented and nonpigmented groups since it was observed that the presence of metals in the media could influence pigmentation and spore production.

Microorganisms isolated from mature oak trees. The number of bacteria on both groups of mature trees increased over the season up to a maximum in November (Table 4), and there was a significant reduction in the number isolated from oaks at the polluted rather than the control site. In July, the total number of bacteria present was uncertain because extensive fungal growth had clearly suppressed bacterial colonies on TSA at lower dilutions, and

TABLE 3. *Mean numbers of microorganisms per square centimeter isolated on TSA with (+M) and without (−M) heavy metals from leaves of oak saplings uncontaminated (C) and dosed with heavy metals (D)*

Microorganism(s)	Treatment	Isolation medium	Mean no. per cm^2			Statistical significance of difference[a]					
						Treatments		Sampling times		Interactions	
			May	July	Sept.	No.	% Tolerant	No.	% Tolerant	No.	% Tolerant
Total bacteria × 10^{-3}	C	−M	0.18	20	94	NS	—	***	—	NS	—
		+M	0	0	0						
	D	−M	0.06	8	110						
		+M	0	0	0						
Total filamentous fungi[b] × 10^{-2}	C	−M	0.09	49	38	NS	NS	***	NS	NS	NS
		+M	0.007	0	1.4						
	D	−M	0.12	34	57						
		+M	0.007	0.11	12						
Cladosporium spp. × 10^{-2}	C	−M	0.07	48	35	NS	NS	***	NS	NS	NS
		+M	0.007	0	0						
	D	−M	0.10	34	54						
		+M	0.007	0.11	2.1						
A. pullulans × 10^{-2}	C	−M	0.03	14	5.0	NS	***	**	***	NS	***
		+M	0.07	1.8	0						
	D	−M	0.007	16	42						
		+M	0.02	6.0	13						
Total yeasts[b] × 10^{-3}	C	−M	0.03	31	13	NS	NS	***	NS	NS	NS
		+M	0.0007	0.05	0.07						
	D	−M	0.01	14	14						
		+M	0	0.25	1.4						
Nonpigmented yeasts[b] × 10^{-3}	C	−M	0.03	10	7.6	NS		***		NS	
	D	−M	0.009	12	11						
Pigmented yeasts[bc] × 10^{-3}	C	−M	0.002	21	5.0	*		***		**	
	D	−M	0.001	1.5	2.7						

[a] Calculated for variance ratios derived from a two-way analysis of variance (repeated-measures design) of total number of isolates and percentage of tolerant isolates. NS, Not significant ($P > 0.05$); *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$; —, no tolerant bacteria isolated.

[b] Excluding *A. pullulans*.

[c] Mostly *S. roseus*.

the remaining plates had been inoculated at too high a dilution to allow accurate assessment. To ascertain that the reduction in bacterial isolates from the polluted site was not attributable to fungal suppression on dilution plates, a similar experiment was conducted during October of the following year. On this occasion bacteria were isolated on TSA amended with cycloheximide and nystatin (50 μg ml^{-1} each) which effectively suppressed growth of fungi. Again, however, far greater numbers of bacteria were isolated from the oaks at the control site (5,800 ± 5,000/cm^2) compared with the polluted oaks (42 ± 6/cm^2). No bacteria were isolated by using metal-supplemented TSA at any time. The composition of the bacterial flora was somewhat varied, but at both sites, *Erwinia* spp. tended to predominate in November 1978.

The total number of fungi isolated also increased over the season, and, as on the saplings, *Cladosporium* spp. tended to predominate among the filamentous forms. A significantly greater proportion of the total filamentous fungi, *Cladosporium* spp., and *A. pullulans* from the polluted oaks were able to grow on metal-supplemented TSA compared with those isolated from the control site. There was also a significant interaction between sites and sampling times in terms of the percentage of metal-tolerant filamentous fungi isolated; in May, July, and September, none were isolated at all from the oaks at the control site, and only in November were a few tolerant isolates recovered from here. The polluted oaks also supported greater numbers of *Cladosporium* spp. and *A. pullulans* (i.e., as measured on metal-free TSA) compared with those at the control site. Conversely, there was a reduction in the total numbers of yeasts (both pigmented and nonpigmented, excluding *A. pullulans*) at the polluted rather than the control

TABLE 4. *Mean numbers of microorganisms per square centimeter isolated on TSA with (+M) and without (−M) heavy metals from leaves of mature oak trees at the control (C) and polluted (P) sites*

Microorganism(s)	Site	Isolation medium	Mean no. per cm²					Statistical significance of difference[a]					
								Sites		Sampling times		Interactions	
			May	July	Sept.	Nov. (leaves)	Nov. (litter)	No.	% Tolerant	No.	% Tolerant	No.	% Tolerant
Total bacteria × 10^{-3}	C	−M	0.04	?[b]	2.7	160	24	*	—	***	—	NS	—
		+M	0	0	0	0	0						
	P	−M	0.03	?[b]	0.57	0.21	37						
		+M	0	0	0	0	0						
Total filamentous fungi[c] × 10^{-2}	C	−M	0.12	3.6	13	25	49	NS	***	***	***	NS	***
		+M	0	0	0	0.09	1.6						
	P	−M	0.24	10	26	25	32						
		+M	0.06	3.5	44	9.2	19						
Cladosporium spp. × 10^{-2}	C	−M	0.08	3.6	11	17	1.4	*	***	**	NS	NS	NS
		+M	0	0	0	0.06	0.007						
	P	−M	0.19	10	26	23	27						
		+M	0.06	3.5	44	9.2	8.5						
A. pullulans × 10^{-2}	C	−M	0.24	86	180	37	24	***	**	***	NS	NS	NS
		+M	0.06	2.5	0.7	3.2	0.57						
	P	−M	0.44	630	720	61	180						
		+M	0.20	390	610	53	165						
Total yeasts[c] × 10^{-2}	C	−M	0.01	16	150	960	290	**	NS	***	*	***	*
		+M	0.007	0	0	1.8	0.75						
	P	−M	0.20	6.7	11	0	100						
		+M	0.03	0	0	1.4	73						
Nonpigmented yeasts[c] × 10^{-2}	C	−M	0.007	15	150	930	270	**		***		***	
	P	−M	0.17	5.7	11	0	98						
Pigmented yeasts[cd] × 10^{-2}	C	−M	0.007	0.99	0	30	20	*		*		*	
	P	−M	0.03	0.92	0	0	5.0						

[a] Calculated for variance ratios derived from a two-way analysis of variance (repeated-measures design) of total number of isolates and percentage of tolerant isolates. NS, Not significant ($P > 0.05$); *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$; —, no tolerant bacteria isolated.

[b] ?, Numbers present uncertain.

[c] Excluding *A. pullulans*.

[d] Mostly *S. roseus*.

site, and there was no overall difference between the two sites in terms of the percentage of metal-tolerant isolates. However, these data for the yeasts should be treated with some degree of caution since the very large numbers of *Aureobasidium* spp. and *Cladosporium* spp. tended to suppress yeast colonies on the dilution plates.

Direct observation of fungal hyphae. A two-way analysis of variance (repeated-measures design) on the data for length of fungal hyphae (Table 5) indicated that there was no significant difference between uncontaminated and dosed saplings ($P > 0.05$), although the difference between sampling times was significant at $P < 0.05$, and the interaction between treatments and sampling times was significant at $P < 0.01$. During May, little hyphal growth had occurred on the leaves of either group of saplings, but in July there was far greater fungal colonization on the controls than on plants sprayed with heavy metals. In September, however, there was little difference between either site. A paired *t* test indicated that there was no significant difference on the controls between upper and lower surfaces ($P > 0.05$), whereas on dosed plants, there was significantly greater colonization of the lower surface ($P < 0.05$).

Greater mycelial colonization occurred on the mature oak trees over the course of the season ($P < 0.001$), but there was no significant difference between the length of hyphae on the control oaks and that at the polluted site ($P > 0.05$) nor any interaction between sites and sampling times ($P > 0.05$; Table 6). Greater colonization took place on the lower rather than the upper surfaces of the oaks at the polluted site ($P <$

TABLE 5. *Mean lengths of fungal hyphae (± the standard error) observed on leaf surfaces of oak saplings uncontaminated and dosed with heavy metals*[a]

Sampling time	Length (mm/mm²)			
	Uncontaminated		Dosed	
	Upper	Lower	Upper	Lower
May	0.032 ± 0.032	0.032 ± 0.032	0.032 ± 0.032	0.25 ± 0.16
July	4.1 ± 0.98	0.79 ± 0.66	0.064 ± 0.032	0.095 ± 0.055
September	0.35 ± 0.03	1.5 ± 0.78	0.25 ± 0.06	2.6 ± 1.6

[a] See text for statistical analysis.

TABLE 6. *Mean lengths of fungal hyphae (± the standard error) observed on leaf surfaces of mature oak trees at the control and the polluted sites*[a]

Sampling time	Length (mm/mm²)			
	Control Site		Polluted Site	
	Upper	Lower	Upper	Lower
May	0.032 ± 0.032	0	0	0
July	1.1 ± 1.0	0.095 ± 0.095	0.38 ± 0.33	0.032 ± 0.032
September	3.3 ± 1.0	0.22 ± 0.13	0.35 ± 0.16	1.6 ± 1.4
November (leaves)	2.8 ± 0.74	2.8 ± 2.7	0.32 ± 0.03	0.60 ± 0.19
November (litter)	1.9 ± 0.83	1.2 ± 0.89	0.32 ± 0.22	3.4 ± 0.32

[a] See text for statistical analysis.

0.05), whereas at the control site, there was no significant difference between surfaces ($P > 0.05$).

DISCUSSION

The chemical nature of the metals sprayed onto the saplings was probably similar to that of the metal pollutants at Avonmouth (1). Dosing the oak saplings with zinc, lead, and cadmium at levels comparable to or greater than those at the polluted site generally appears to have had little influence on most groups of microorganisms. This is in agreement with studies on cabbage leaves in which artificial metal contamination had little effect on naturally infecting microorganisms (7). The major exception to this in the present study were the pigmented yeasts (chiefly *S. roseus*) which appeared sensitive to the effects of dosing and to natural pollution. Numbers of pigmented yeasts were also significantly reduced on cabbages exposed to heavy metal pollution in this area (7), so from the dosing study, it is reasonable to assume that this reduction was at least partly attributable to heavy metals rather than to some other pollutant.

Mycelial proliferation, estimated by direct observation, and total viable counts of fungal propagules, isolated by leaf washing, appeared to be little affected by heavy metal pollution. Not only was a greater proportion of the predominant *Cladosporium* spp. and *A. pullulans* on the polluted oaks at Avonmouth tolerant to heavy metals, but significantly greater numbers of these organisms were isolated from these polluted oaks compared with the oaks at the control site. The present investigation formed part of a scanning electron microscope study of the relationship between the leaf surface mycroflora of these oaks at Avonmouth and pollutant dust particles (5). Certain types of pollutant dust particles supported a greater density of fungal propagules than the adjacent leaf surface, and it was suggested that such stimulation may be attributable to the occurrence of nutrients within the particles, the beneficial effects of which might outweigh the toxic effect of any heavy metals. This may also explain the increase in numbers of these particularly metal-tolerant fungi on the polluted oaks, compared with those at the control site. It was unclear from the scanning electron microscopy studies (5) whether the metal pollutants were present on oak leaves in a finely divided form or as a few concentrated sources possibly not even available to the microflora. However, since a high proportion of *Cladosporium* spp. and *A. pullulans* at the polluted site was tolerant to metals in vitro, it seems likely that a similarly high proportion were in contact with the metals on the leaf surface. Had there been little difference in metal tolerance between the microflora of the control and polluted oaks, one would have suspected that few of the latter were in contact with any of the metals. Hence, it is reasonable to conclude that the metals were finely distributed on the phylloplane.

The absence of bacteria on plates containing zinc, lead, and cadmium supports previous evi-

dence that the bacterial phylloplane flora is inherently less tolerant to heavy metals than the fungi (4). Nevertheless, there was little reduction in total numbers of bacteria (as recorded on metal-free TSA) on plants dosed with levels of zinc, lead, and cadmium at levels at least as high as those encountered on the polluted oaks. For this reason, it seems unlikely that the reduction in the bacterial flora of the polluted oaks at Avonmouth compared with those at the control site was directly attributable to heavy metal pollution. Other pollutants at Avonmouth such as sulfur dioxide and oxides of nitrogen could be of greater significance. Furthermore, such pollutants may interact synergistically with the heavy metals present, thus increasing their relative toxicities.

The present study therefore indicates that the phylloplane microflora has a generally high capacity to survive under conditions of considerable heavy metal contamination, although there appear to be differences in metal tolerance between different groups of microbes.

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LITERATURE CITED

1. **Allen, G., G. Nickless, B. Wibberley, and J. Pick.** 1974. Heavy metal particle characterisation. Nature (London) **252**:571–572.
2. **Babich, H., and G. Stotzky.** 1974. Air pollution and microbial ecology. Crit. Rev. Environ. Control **4**:353–421.
3. **Babich, H., and G. Stotzky.** 1978. Effects of cadmium on the biota: influence of environmental factors. Adv. Appl. Microbiol. **23**:55–117.
4. **Bewley, R. J. F.** 1979. The effects of zinc, lead, and cadmium pollution on the leaf surface microflora of *Lolium perenne* L. J. Gen. Microbiol. **110**:247–254.
5. **Bewley, R. J. F., and R. Campbell.** 1978. Scanning electron microscopy of oak leaves contaminated with heavy metals. Trans. Br. Mycol. Soc. **71**:508–511.
6. **Dickinson, C. H., B. Austin, and M. Goodfellow.** 1975. Quantitative and qualitative studies of phylloplane bacteria from *Lolium perenne*. J. Gen. Microbiol. **91**:157–166.
7. **Gingell, S. M., R. Campbell, and M. H. Martin.** 1976. The effect of zinc, lead and cadmium pollution on the leaf surface microflora. Environ. Pollut. **11**:25–37.
8. **Jensen, V.** 1971. The bacterial flora of beech leaves, p. 463–469. *In* T. F. Preece and C. H. Dickinson (ed.), Ecology of leaf surface microorganisms. Academic Press, London.
9. **Last, F. T.** 1955. Seasonal incidence of *Sporobolomyces* on cereal leaves. Trans. Br. Mycol. Soc. **38**:221–229.
10. **Last, F. T., and R. C. Warren.** 1972. Non-parasitic microbes colonising green leaves: their form and function. Endeavour **31**:143–150.
11. **Little, P.** 1973. A study of heavy metal contamination of leaf surfaces. Environ. Pollut. **5**:159–172.
12. **Little, P., and M. H. Martin.** 1972. A survey of zinc, lead, and cadmium in soil and natural vegetation around a smelting complex. Environ. Pollut. **3**:241–254.
13. **Pearce, S. C.** 1965. Biological statistics: an introduction. McGraw-Hill Book Co., London.
14. **Rovira, A. D., E. I. Newman, H. J. Bowen, and R. Campbell.** 1974. Quantitative assessment of the rhizoplane microflora by direct microscopy. Soil Biol. Biochem. **6**:211–216.
15. **Winer, B. J.** 1971. Statistical principles in experimental design. McGraw-Hill Book Co., New York.